REMARKS

The specification and Claims 14, 20, and 26 have been amended to correct various typographical errors. No question of new matter arises and entry of the amendments is respectfully requested.

Applicants submit herewith Figures 8A, 8B, which have been amended to correct typographical errors, and Figures 11A and 13A which have been amended to more clearly define the MCS and GS Linker. The proposed changes are shown in red ink. No new matter has been added. Applicants respectfully request approval of these drawing changes.

Applicants respectfully submit that this application is in condition for allowance and request favorable consideration.

If any points remain at issue which can best be resolved by way of a telephonic or personal interview, the Examiner is kindly requested to contact the undersigned attorney at the local telephone number listed below.

Respectfully submitted,

PIPER RUDNICK MARBURY & WOLFE, LLP

Amy L Miller

Steven B. Kelber
Registration No: 30,073
Attorney of Record

Amy L. Miller
Registration No: 43,804

1200 Nineteenth Street, N.W.
Washington, D.C. 20036-2412
Telephone No.: (202) 861-3900
Facsimile No.: (202) 223-2085

SERIAL NO. 09/654,499

DOCKET NO.: 4085-226-27

MARKED-UP COPY OF PARAGRAPHS, AS AMENDED

Replacement for first paragraph, at page 2, lines 1-8:

--The signaling pathway and final cellular response that result from GPCR stimulation depends on the specific class of G-protein with which the particular receptor is coupled (Hamm, "The many faces of G-Protein Signaling." J. Biol. Chem., 273:669-672 (1998)). For instance, coupling to the Gs class of G-proteins stimulates cAMP production and activation of Protein Kinase A and C pathways, whereas coupling to the Gi class of G-proteins down regulates cAMP. Other second messenger systems as calcium, [phosphlipase] phospholipase C, and phosphatidylinositol 3 may also be utilized. As a consequence, GPCR signaling events have predominantly been measured via quantification of these second messenger products.--

Replacement for the first full paragraph, at page 4, lines 3-6:

--Efforts such as the Human Genome Project are identifying new GPCRs ("orphan" receptors) whose physiological roles and ligands are unknown. It is estimated that several thousand GPCRs exist in the human genome. [Of the 250 GPCRs identified to date, only 150 have been associated with ligands.]--

Replacement for the first paragraph, at page 10, lines 1-9:

--FIGURE 3. Interaction of activated receptor β2AR and arrestin can be measured by β-galactosidase complementation. Figure 3A shows a time course of β–galactosidase activity in

response to agonist (-)isoproterenol stimulation in C2 expressing β2AR-βgalΔα (β2AR alone, in expression vector pICAST ALC), or C2 clones, and a pool of C2 co-expressing β2AR-βgalΔα and βArr2-βgalΔω (in expression vectors pICAST ALC and pICAST OMC). Figure 3B shows a time course of [β galactosidase] <u>β–galactosidase</u> activity in response to agonist (-)isoproterenol stimulation in C2 cells expressing β2AR alone (in expression vector pICAST ALC) and C2 clones co-expressing β2AR and βArr1 (in expression vectors ICAST ALC and pICAST OMC).--

Replacement for second full paragraph, at page 11, lines 7-12:

--FIGURE 7. Agonist stimulated cAMP response in C2 cells co-expressing Dopamine receptor D1 (D1-βgalΔα) and β-arrestin-2 (βArr2-βgalΔω). The clone expressing βArr2-βgalΔω (Arr2 alone) was used as a negative control in the assay. Cells expressing D1-βgalΔα in addition to βArr2-βgalΔω responded [agonist treatment] <u>to treatment with agonist</u> (3-hydroxytyramine hydrochloride at 3 μM) . D1(PIC2) or D1(PIC3) designate D1 in expression vector pICAST ALC2 or pICAST ALC4, respectively.--

Replacement for fifth full paragraph, at page 14, lines 19-20:

--FIGURE 23. A schematic depicting the method of the invention, which shows that two inactive mutants [that] become active when they interact.--

Replacement for second paragraph, at page 15, lines 3-15:

--The present invention provides a method to interrogate GPCR function and pathways. The G-protein-coupled superfamily continues to expand rapidly as new receptors are discovered through automated sequencing of cDNA libraries or genomic DNA. It is estimated that several

thousand GPCRs may exist in the human genome[, as many as 250 GPCRs have been cloned and only as few as 150 have been associated with ligands]. <u>Only a portion have been cloned and even fewer have been associated with ligands.</u> The means by which these, or newly discovered orphan receptors, will be associated with their cognate ligands and physiological functions represents a major challenge to biological and biomedical research. The identification of an orphan receptor generally requires an individualized assay and a guess as to its function. The interrogation of a GPCR's signaling behavior by introducing a replacement receptor eliminates these prerequisites because it can be performed with and without prior knowledge of other signaling events. It is sensitive, rapid and easily performed and should be applicable to nearly all GPCRs because the majority of these receptors should desensitize by a common mechanism.--

Replacement for fourth paragraph, at page 16, lines 13-20:

--The present invention involves a method for monitoring protein-protein interactions in GPCR pathways as a complete assay using ICAST™ (Intercistronic Complementation Analysis Screening Technology as disclosed in pending U.S. patent application serial no. [053,164] <u>053,614</u>, filed April 1, 1998, the entire contents of which are incorporated herein by reference). This invention enables an array of assays, including GPCR binding assays, to be achieved directly within the cellular environment in a rapid, non-radioactive assay format amenable to high-throughput screening. Using existing technology, assays of this type are currently performed in a non-cellular environment and require the use of radioisotopes.--

Replacement for fifth paragraph, at page 16, line 21-page 17, line 11:

The present invention combined with Tropix ICAST™ and Advanced Discovery Sciences™ technologies, e.g., ultra high-throughput screening, provide highly sensitive cell-based methods for interrogating GPCR pathways which are amendable to high-throughput screening (HTS). These methods are an advancement over the invention disclosed in U.S. Patent 5,891,646, which relies on microscopic imaging of GPCR components as fusion with Green-fluorescent-protein. Imaging techniques are limited by low-throughput, lack of thorough quantification and low signal to noise ratios. Unlike yeast-based-2-hybrid assays used to monitor protein/protein interactions in high-throughput assays, the present invention is applicable to a variety of cells including mammalian cells, plant cells, protozoa cells such as E. coli and cells of invertebrate origin such as yeast, slime mold (*Dictyostelium*) and insects; detects interactions at the site of the receptor target or downstream target proteins rather than in the nucleus; and does not rely on indirect read-outs such as transcriptional activation. The present invention provides assays with greater physiological relevance and fewer false [negatives] positives.

Replacement for first full paragraph, at page 17, lines 12-22:

--Advanced Discovery Sciences™ is in the business of offering custom-developed screening assays optimized for individual assay requirements and validated for automation. These assays are designed by HTS experts to deliver superior assay performance. Advanced Discovery Sciences[']™ custom assay development service encompasses the design, development, optimization and transfer of high performance screening assays. Advanced Discovery Sciences™ works to design new assays or convert existing assays to ultra-sensitive

luminescent assays ready for the rigors of HTS. Among some of the technologies developed by Advanced Discovery Sciences™ are the cAMP-Screen™ immunoassay system. This system provides ultrasensitive determination of cAMP levels in cell lysates. The cAMP-Screen™ assay utilizes the high-sensitivity chemiluminescent alkaline phosphatase (AP) substrate CSPD® with Sapphire-II™ luminescence enhancer.--

Replacement for first paragraph, at page 18, lines 2-14:

--GPCR activation can be measured through monitoring the binding of ligand-activated GPCR by an arrestin. In this assay system, a GPCR, e.g. [β adrenergic] β-adrenergic receptor [(β 2AR)] (β2AR) and a [β arrestin] β-arrestin are co-expressed in the same cell as fusion proteins with [β gal] β-gal mutants. As illustrated in Figure 1, the β2AR is expressed as a fusion protein with Δα form of [β gal] β-gal mutant (β2ADRΔα) and the [b arrestin] β-arrestin as a fusion protein with the Δω mutant of [β gal] β-gal (β-ArrΔω). The two fusion proteins exist inside of a resting (or un-stimulated) cell in separate compartments, i.e. membrane for GPCR and cytosol for arrestin, and they [can not] cannot form an active [b galactosidase] β-galactosidase enzyme. When such a cell is treated with an agonist or a ligand, the ligand-occupied and activated receptor will become a high affinity binding site for Arrestin. The interaction between an activated β2ADRΔα and β–ArrΔω drives the [β gal gal] β-gal mutant complementation. The enzyme activity can be measured by using an enzyme substrate, which upon cleavage releases a product measurable by colorimetry, fluorescence, chemiluminescence (e.g. Tropix product GalScreen[TM] ®).--

Replacement for the first full paragraph, at page 19, lines 2-12:

--3. In the last step, the cells expressing both β2ADRΔα and βArr2Δω were tested for response by agonist/ligand stimulated [β galactosidase] β-galactosidase activity. Triplicate samples of cells were plated at 10,000 cells in 100 microliter volume into a well of 96-well culture plate. Cells were cultured for 24 hours before assay. For an agonist assay (Figures 3 and 4), cells were treated with variable concentrations of agonist, for example, (-) isoproterenol, procaterol, dobutamine, [terbutiline] terbutaline or [L-L-phenylephrine] L-phenylephrine for 60 min at 37° C. The induced [β galatosidase] β-galactosidase activity was measured by addition of Tropix GalScreen[TM] ® substrate (Applied Biosystems) and luminescence measured in a [Tropix TR717TM] TR717™ luminometer (Applied Biosystems). For antagonist assay (Figure 5), cells were pre-incubated for 10 min in fresh medium without serum in the presence of ICI-118,551 or propranolol followed by addition of 10 [micro molar] micromolar (-) isoproterenol.--

SERIAL NO. 09/654,499

DOCKET NO.: 4085-226-27

MARKED-UP COPY OF AMENDED CLAIMS

14. (Amended) A method according to Claim 10, wherein the cell expresses a GPCR, a β-adrenergic [GPCR] receptor.

20. (Amended) A method of screening a plurality of cells for those cells which contain a G-protein-coupled receptor (GPCR) responsive to a GPCR agonist, comprising:

a) providing a plurality of cells, said cells containing a conjugate comprising a β-arrestin protein as a fusion protein with a reporter enzyme;

b) exposing the cells to a GPCR agonist; and

c) detecting enzymatic activity of the reporter enzyme;

wherein an increase in enzymatic activity after exposure of the cell to the GPCR agonist indicates β-arrestin protein binding to a GPCR, thereby indicating that the cell contains a GPCR responsive to said GPCR agonist.

26. (Amended) A substrate according to Claim 24, wherein the substrate is made of organic compounds or synthetic polymers, or from a material selected from glass, plastic, ceramic, semiconductor, silica, fiber optic, diamond, biocompatible monomer and biocompatible polymer materials.

ABSTRACT

Methods for detecting G-protein coupled receptor (GPCR) activity; methods of assaying GPCR activity; and methods of screening for GPCR ligands, G-protein-coupled receptor kinase (GRK) activity, and compounds that interact with components of the GPCR regulatory process are described.

a 15